Two Guanylate Cyclase Activating Proteins in Medaka Retina

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Two Guanylate Cyclase Activating Proteins in Medaka Retina

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Guanylate cyclase activating protein (GCAP1 and 2) is a Ca^{2+}-sensitive regulator of the retinal membrane guanylate cyclases (GCs). In mammalian retina, GCAP1 is localized in cones and GCAP2 is present in rods, cones and other retinal cells. Here we isolated two kinds of cDNAs encoding putative medaka GCAPs (OlGCAP1 and OlGCAP2). Sequence analysis and characterization of recombinant proteins indicate that OlGCAP1 and 2 are closely related to mammalian GCAP1 and 2, respectively, and that OlGCAP1 and 2 appear to regulate GCs in a manner similar to that of mammalian GCAPs. However, *in situ* hybridization and immunocytochemistry suggest that both OlGCAP1 and 2 coexist mainly in rods, and that OlGCAP1, but not OlGCAP2, is present in the inner nuclear layer and ganglion cell layer, indicating that localization of these medaka GCAPs is totally different from that of mammalian GCAPs. The Ca^{2+}-feedback system in vertebrate retinal phototransduction may be evolved in the expression of GCs and GCAPs in photoreceptors.

KEYWORDS: ganylate cyclase activating protein (GCAP); cDNA cloning; *in situ* hybridization; immunocytochemistry; medaka (*Oryzias latipes*)

INTRODUCTION

In rod photoreceptors, photoactivation of rhodopsin leads to the closure of cGMP-gated cation channels, which causes hyperpolarization of the membrane potential and a decrease of the intracellular Ca^{2+} concentration (Baylor *et al.*, 1996; Koutalos *et al.*, 1996; Stryer *et al.*, 1996; Molday *et al.*, 1998). The lowering of the Ca^{2+} concentration activates membrane-bound guanylate cyclases (GCs), and accelerates the recovery of photoreceptors to the dark state (Koch *et al.*, 1988; Pugh *et al.*, 1997). This Ca^{2+}-sensitive GC activation is due to Ca^{2+}-binding proteins, termed guanylate cyclase activating proteins (GCAPs). Ca^{2+}-free forms of GCAPs activate photoreceptor GCs. GCAP has two isoforms, GCAP1 and 2 (Dizhoor *et al.*, 1994, 1995; Gorczyca *et al.*, 1994, 1995; Palczewski *et al.*, 1994). The localization of these GCAP isoforms has been investigated by immunocytochemistry, suggesting that GCAP1 is mainly located in cone photoreceptors and GCAP2 is localized in rod and cone photoreceptors and other retinal cells (Cuenca *et al.*, 1998; Howes *et al.*, 1998; Kachi *et al.*, 1999). Haeseleer *et al.* (1999) have also reported the presence of the third GCAP isoform, GCAP3, which appears to have a property similar to GCAP1 and 2 in the regulation of GCs.

Although mammalian GCAPs have been studied vigorously, non-mammalian GCAPs have not been investigated at all, except for the deduced amino acid sequences of frog and chicken GCAPs (Palczewski *et al.*, 1994; Semple-Rowland *et al.*, 1999). The characterization of non-mammalian GCAPs is essential to reveal the evolution process of a Ca^{2+}-feedback system in phototransduction of vertebrate retinal photoreceptors. We have previously reported that two kinds of GCs (OlGC-R1 and -R2) are coexpressed in rods, whereas OlGC-C is expressed in cones (Hisatomi *et al.*, 1999). In this study, we isolate two kinds of cDNAs encoding the putative GCAPs (OlGCAP1 and 2) from a medaka retinal cDNA library, and compare the properties and distribution of these proteins with mammalian counterparts.

EXPERIMENTAL PROCEDURES

Isolation of cDNAs encoding OlGCAP1 and OlGCAP2. We prepared degenerate oligonucleotide primers, GCAP-F1 and GCAP-R1 corresponding to the amino acid sequences, QWYKKF and NGCIDR, respectively (Fig. 1). Two kinds of cDNA fragments encoding medaka (*Oryzias latipes*) GCAPs, OlGCAP1 and OlGCAP2, were amplified by polymerase chain reaction using a retinal cDNA pool as a template (Hisatomi *et al.*, 1997a, 1999b). A medaka retinal cDNA library was screened at high stringency using the amplified fragments as probes (Hisatomi *et al.*, 1997a, 1999b). Positive clones were transformed into plasmids by an EXASSIST-SOLR system (Stratagene), and sequenced according to the cycle sequencing method (Applied Biosystems).

In situ hybridization. The OlGCAP1 (585 bp) and OlGCAP2 (614 bp) cDNA fragments were cloned into a pGEM-3Zf(+) plasmid vector (Promega), and linearized with appropriate endonucleases. Antisense cRNA

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a

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                                          GCAP-F1      EF1
                *                          →
OlGCAP1         MGN-STGSTVDD----------LQAVEMHLWYKKFMTECPSGQLTLHEFKQFFGLKGLME-ANAYIEQM   57
bovine GCAP1    MGNIMDGKSVEE----------LSSTECHQWYKKFMTECPSGQLTLYEFRQFFGLKNLSPWASQYVEQM   59
OlGCAP2         MGQRLSESDPDKE---------IDVADVQEWYKKFVVECPSGTLFMHEFKGFFGVTNNKE-AADYIENM   58
bovine GCAP2    MGQQFSWEEAEE----NGAVGAADAAQLQEWYKKFLEECPSGTLFMHEFKRFFKVPDNEE-ATQYVEAM   64
human GCAP3     MGNGKSIAGDQK---------AVPTQETHVWYRTFMMEYPSGLQTLHEFKTLLGLQGLNQKANKHIDQV   60
Recoverin       MGNSKSGALSKEILEELQLNTKFTEEELSSWYQSFLKESPSGRITRQEFQTIYSKFFPEADPKAYAQHV   69

          EF2                    EF3  GCAP-R1
                                       ←
FRTFDMNKDGYIDFMEYVAALSLVMRGKMEHKLRWYFKLYDVDGNGCIDRHELLNIIKAIRAINGNE---------NQEMT  133
FETFDFNKDGYIDFMEYVAALSLVLKGKVEQKLRWYFKLYDVDGNGCIDRDELLTIIRAIRAINPCS---------DTTMT  131
FRAFDKNGDNTIDFLEYVAALNLVLRGKLEHKLKWTFKMYDKDGSGCIDKTELLEIVESIYRLKKAC--HGELDEECNLLT  141
FRAFDTNGDNTIDFLEYVAALNLVLRGTLEHKLKWTFKIYDKDRNGCIDRQELLDIVESIYKLKKACSVEVEAEQQGKLLT  145
YNTFDTNKDGFVDFLEFIAAVNLIMQEKMEQKLKWYFKLYDADGNGSIDKNELLDMFMAVQALNGQQ-----------TLS  135
FRSFDANSDGTLDFKEYVIALHMTSAGKTNQKLEWAFSLYDVDGNGTISKNEVLEIVTAIFKMISPE---DTKHLPEDENT  151

          EF4
AEEFTNNVFDRIDVNGDGELSLEEFVEGARSDEDFMEVMMKSLDLRHIVAMIHNRRHSV                        188
AEEFTDTVFSKIDVNGDGELSLEEFMEGVQKDQMLLDTLTRSLDLTRIVRRLQNGEQDEEGASGRETEAAEADG        205
PDQVVDRIFELVDENGDGELSLDEFIDGARRDKWVMKMLQMDVNPGDWINE-RRCSEDF                        196
PEEVVDRIFLLVDENGDGQLSLNEFVEGARRDKWVMKMLQMDLNPSSWISQQRRKSAMF                        204
PEEFINLVFHKIDINNDGELTLEEFINGMAKDQDLLEIVYKSFDFSNVLRVICNGKQPDMETDSSKSPDKAGLGKVKMK  209
PEKRAEKIWGFFGKKDDDKLTEKEFIEGTLANKEILRLIQFEPQKVKEKLKEKKL                           202
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b

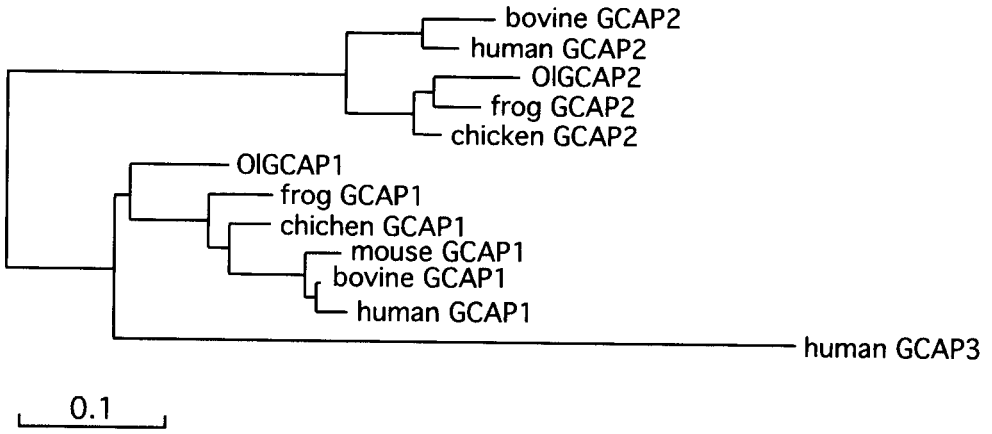

Fig. 1. (a) The deduced amino acid sequences of OlGCAP1 and OlGCAP2 aligned with the sequences of bovine GCAP1 (P46065), bovine GCAP2 (P51177), human GCAP3 (Haeseleer *et al*, 1999) and bovine recoverin (P21457). Bold lines above the sequences show EF-hand motifs, and arrows indicate the amino acid sequences corresponding to GCAP-F1 and GCAP-R1 primers used for the amplification of GCAP cDNA fragments. An asterisk represents putative *N*-myristoylation sites (Toweler *et al.*, 1988; Rocque *et al.*, 1993). The nucleotide sequences in this figure have been submitted to the GeneBank/EMBL/DDBJ nucleotide databeses with accession numbers AB055969 (OlGCAP1) and AB055970 (OlGCAP2). (b) An NJ tree calculated from the amino acid sequences of vertebrate GCAPs. Bar indicates 10% replacement of an amino acid per site. The sequence data used in the present analysis were taken from EMBL and Swiss-Prot database (accession numbers in parentheses), and from the literature: human GCAP1 (Palczewski *et al.*, 1994), GCAP2 (Q9UMX6) and GCAP3 (Haeseleer *et al.*, 1999), mouse GCAP1 (Palczewski *et al.*, 1994), bovine GCAP1 (P46065) and GCAP2 (P51177), chicken GCAP1 (P79880) and GCAP2 (P79881), and frog GCAP1 (O73761) and GCAP2 (O73762).

riboprobes were synthesized by run-off transcription from the T7 promoter with digoxigenin-UTP, as recommended in the manufacturer's protocol (Boehringer Mannheim). The preparation of medaka retinal cryosections and methods for *in situ* hybridization, using 0.1 μg/ml (final concentration) of OlGCAP1 and 2 cRNAs as probes, were as described previously (Raymond *et al.*, 1993; Hisatomi *et al.*, 1996, 1997a, 1999b).

Expression of recombinant medaka GCAPs. Expression and isolation of the recombinant medaka GCAPs were carried out according to those of bullfrog S-modulin and mammalian GCAP2 with modifications as follows (Hisatomi *et al.*, 1997b; Olshevskaya *et al*, 1997). The coding regions of OlGCAP1 and OlGCAP2 were inserted between the NcoI and XhoI sites (OlGCAP1) or between the NcoI and BamHI sites (OlGCAP2) of a pET16b vector (Novagen). The plasmids were introduced into the BL21 (DE3) cells carrying a pBB131 plasmid, an expression vector of yeast *N*-myristoyl transferase (kindly provided by Prof. Jeffrey I. Gordon), and the recombinant proteins were expressed by the addition of 1 mM isopropyl-1-thio-β-D-galactopyranoside into the culture medium. Cells were harvested by centrifugation (2,700 × g for 15 min), disrupted by five cycles of ultrasonication in the reconstitution buffer (5 mM β-mercaptomethanol, 1 mM EDTA, 100 μM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 20 mM Tris-HCl, pH 7.5), and centrifuged at 6,500 × g for 30 min. The recombinant GCAPs in the insoluble fraction were solubilized by homogenization in 8 M urea buffer (8 M urea, 100 mM β-mercaptomethanol, 1 mM EDTA, 100 μM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 20 mM Tris-HCl, pH 7.5), followed by the stepwise dialysis (3h each) against the reconstitution buffer containing 4 M, 2 M, 1 M, and 0 M urea at 4°C. The centrifugal supernatants from the dialysate were applied to a Hi-Trap Q (Pharmacia) column pre-equilibrated with the reconstitution buffer. The recombinant medaka GCAPs were eluted with a linear gradient 0.2–0.8 M NaCl in the reconstitution buffer, and used for the spectroscopic measurements and for the immunization of mice as antigens. Sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE) showed that the purity of these GCAPs was more than 95% (data not shown).

Spectroscopic measurements. Fluorescence emission spectra of recombinant GCAPs were recorded from 300 to 400 nm by a fluorescence spectrophotometer (Hitachi, F-4500) with an excitation wavelength at 290 nm in mixtures containing 0.5 or 1 μM recombinant proteins, 100 mM KCl, 5 mM β-mercaptoethanol, 1 mM EGTA, $CaCl_2$ and 100 mM HEPES (pH 7.5). Free Ca^{2+} concentrations were adjusted by addition of $CaCl_2$ (Tsien *et al.*, 1989).

Western blot analysis and Immunocytochemistry. Retinas were removed from dark-adapted medakas under dim red light and shaken for 1 min in the HEPES buffer (115 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.5) containing 35% (w/w) sucrose. The suspension was centrifuged for 15 min at 13,000 rpm and the supernatant was diluted by an addition of 2 volumes of the HEPES buffer. ROS (rod outer segments) were collected by centrifugation and immersed into SDS sample buffer. SDS-PAGE was carried out according to the standard method using 15% polyacrylamide mini-gels (Hisatomi *et al.*, 1999; Imanishi *et al.*, 1999). Proteins were transferred to PVDF membranes (Bio-Rad), and incubated with a 1000-fold dilution of antisera for 2 h. Alkaline phosphatase-conjugated anti-mouse IgG was reacted as recommended by the manufacturer (Vector Laboratories), and antibody binding was visualized with NBT/BCIP (Boehringer Mannheim).

Immunocytochemical procedures were carried out as described by Imanishi *et al.* (1999). Briefly, cryosections (5 μm) of the medaka eye cup were incubated with 100-fold dilution of the antisera, washed with PBS, and developed with Histofine SAB-MO kit (Nichirei). Localization of GCAPs was visualized using Nomarski optics (Olympus-BX50).

RESULTS AND DISCUSSION

Isolation of cDNAs and the deduced amino acid sequences of medaka GCAPs. Two kinds of medaka (*Oryzias latipes*) cDNAs encoding the putative GCAPs, OlGCAP1 and OlGCAP2, were isolated in our screening. OlGCAP1 and 2 likely consist of 188 and 196 amino acids, respectively (Fig. 1a). Each deduced amino acid sequence possesses a putative *N*-myristoylation site at Gly^2 and four EF-hand motifs. The amino acid sequences of OlGCAP1 and 2 share approximately 60% identity each other but their N and C terminal sequences are considerably different. The sequence of OlGCAP1 is highly homologous to that of bovine GCAP1 (82% identity), but less homologous to those of human GCAP3 (59% identity) and bovine GCAP2 (64% identity). In contrast, OlGCAP2 is much more similar to bovine GCAP2 (81% amino acid identity) in comparison to bovine GCAP1 (59% identity) and human GCAP3 (47% identity). A phylogenetic tree calculated by the neighbor-joining (NJ) method clearly indicates that OlGCAP1 and 2 are closely related to other vertebrate GCAP1s and GCAP2s, respectively (Fig. 1b).

To address whether another GCAP isoform(s) (such as GCAP3) exists in medaka retina, we have thoroughly screened the medaka retinal cDNA library at low stringency using O1GCAP1 and 2 cDNA fragments as probes. But we could not find another GCAP cDNA, suggesting that medaka retina may not have the third GCAP

isoform. Alternatively, the third isoform may have an amino acid sequence considerably different from those of O1GCAP1 and 2, or its content in medaka retina may be very low compared with those of O1GCAP1 and 2.

For the interaction with GCs, amino acid sequences of three regions (S9-E28, E89-D108 and L112-T131) of bovine GCAP1, which are considered as potential contact surfaces to Ret-GC1 (Krylov *et al.*, 2001), are remarkably conserved in OlGCAP1 though there are some substitutions in the first region (Fig. 1a). Also, the amino acid residues in three regions (K29-F48, F78-D113 and V171-N189) of bovine GCAP2 crucial for GC regulation (Olshevskaya *et al.*, 1999) are almost identical to those in the corresponding regions of OlGCAP2 (Fig. 1a). These common characteristics suggest that medaka GCAPs interact with GCs in a manner similar to that of mammalian GCAPs.

For Ca^{2+} binding, three EF-hands (EF_2-4) in medaka GCAPs have proper amino acid residues for coordinating Ca^{2+} like those in mammalian GCAPs, suggesting these EF-hands of medaka GCAPs are presumably capable of binding Ca^{2+}. We also found that, like EF1 of mammalian GCAPs (Dizhoor and Hurley, 1996), EF1s of both OlGCAP1 and 2 appear to lose the ability for the binding of Ca^{2+}. Because the third position (OlGCAP1) or the third and ninth position (OlGCAP2) of EF1 lack oxygen-containing side chain crucial for Ca^{2+} binding, and the first asparatic acid residues of EF1s (OlGCAP1 and 2), which are essential for Ca^{2+} binding (Dizhoor and Hurley, 1996), are substituted by other amino acid residues. These resemblances between mammalian GCAPs and medaka GCAPs suggest that the activation mechanism of GC by medaka GCAPs is similar to that by mammalian GCAPs.

Ca^{2+}-induced conformational change of the recombinant medaka GCAPs. In order to show Ca^{2+} binding to medaka GCAPs, we measured fluorescence spectrum of medaka GCAPs in the presence or absence of Ca^{2+} because conformational changes of some proteins by Ca^{2+} binding can be monitored by measuring its fluorescence emission spectrum (Hisatomi *et al.*, 1997b). In recoverin (another retinal Ca^{2+}-binding protein), tryptophan residues (W31 and W104) near EF1 and EF3 fit snugly around the myristyl group sequestered in the interior of the protein without Ca^{2+}. However, these tryptophan residues are exposed or move apart by Ca^{2+} binding to the protein (Tanaka *et al.*, 1995; Ames *et al.*, 1997). This conformational transition affects its fluorescence emission spectrum (Ray *et al.*, 1992; Hisatomi *et al.*, 1997b; Matsuda *et al.*, 1998). Using *N*-myristoylated medaka GCAPs expressed in *E. coli*, we measured tryptophan emission spectra because medaka GCAPs have two tryptophan residues (W20 and W92 of OlGCAP1, W21 and W93 of OlGCAP2) at the corresponding positions (Fig. 1a). OlGCAP1 showed maximum emission at 337 and 338 nm in the absence (1 nM) or presence (10 μM) of free Ca^{2+}, respectively (Fig. 2a). In the case of OlGCAP2, the maximum emissions were at 340 nm (at 1 nM free Ca^{2+}) and at 343 nm (at 10 μM free Ca^{2+}) (Fig. 2b). Since these red-shifts, though small, were consistently observed under physiological Ca^{2+} concentrations, these changes appear to be significant. However, these red-shifts of the maximum emission were much smaller compared with those of bovine recoverin and bullfrog S-modulin (Ray *et al.*, 1992; Hisatomi *et al.*, 1997b; Matsuda *et al.*, 1998), suggesting that Ca^{2+} induced conformational change in the N-terminal domain of recoverin, referred to as the 'calcium-myristyl switch', does not occur in medaka GCAPs. We note that the 'calcium-myristyl switch' has been suggested not to operate in mammalian GCAPs (Olshevskaya *et al.*, 1997; Hughes *et al.*, 1998). This is another common character between medaka and mammalian GCAPs.

Localization of GCAPs in medaka retina. We also compared localization of medaka GCAPs with mammalian GCAPs. The distributions of the medaka GCAP mRNAs were investigated by *in situ* hybridization, which was

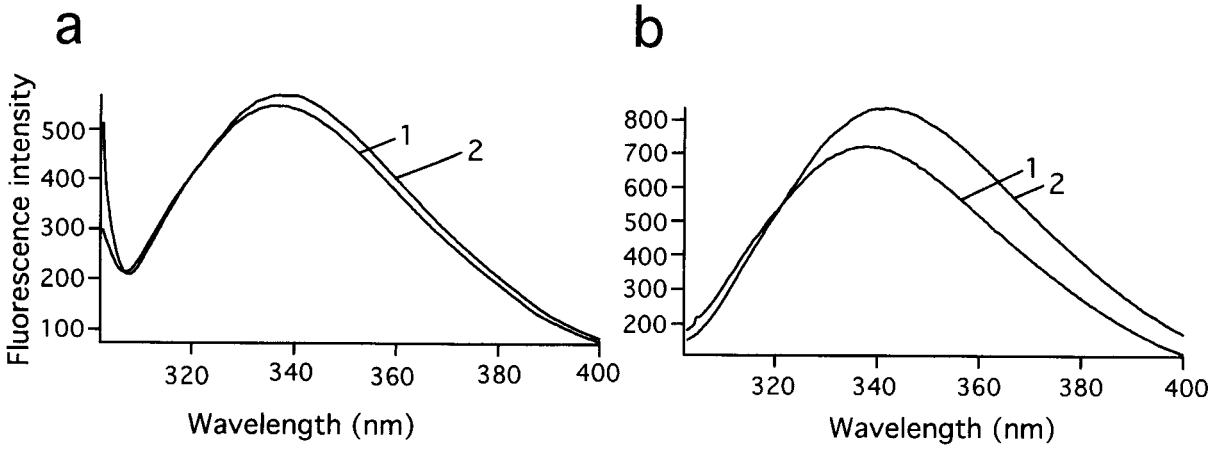

Fig. 2. Fluorescence emission spectra of OlGCAP1 (a) and OlGCAP2 (b) at low (1 nM, curve 1) or high (10 μM, curve 2) free Ca^{2+} concentrations.

performed at high stringency to avoid the cross-hybridization of digoxigenin-conjugated cRNA probes to mRNA of the other GCAP (63% identity for nucleotides). Both of the cRNA probes predominantly recognized the outer nuclear layer in radial sections of medaka retina (Figs. 3a and 3b). These hybridization signals were mainly found in the cell bodies and myoids of rods but scarcely detected in cones. Since almost all rods appear to be positive for both OlGCAP1 and 2 cRNA probes, it is likely that OlGCAP1 and 2 are coexpressed in medaka rods. Difference in signal intensities may suggest that contents of mRNA for O1GCAP1 may be larger than that of O1GCAP2 in medaka rods.

We also used immunocytochemistry to investigate the localization of medaka GCAPs. For this purpose, we raised antisera, anti-OlGCAP1 and anti-OlGCAP2, against recombinant medaka GCAPs expressed in *E. coli*. Each antisera recognized only one protein in medaka ROS homogenates (Fig. 4a). These bands are likely to be the native OlGCAP1 and 2, since they had almost the same mobility as recombinant medaka GCAPs (approximately 23 and 20 kDa, respectively). Together, these observations indicate that each antiserum is specific to its own antigen and shows no cross-reactivity with the other antigen. Under these conditions, immunocytochemistry showed that anti-O1GCAP1 reactivity was mainly observed in rod outer and inner segments (Fig. 4b). Anti-OlGCAP2 reactivity was also found predominantly in rod outer and inner segments (Fig. 4c). These results indicate that both OlGCAP1 and 2 are mainly located in rod photoreceptors. This is consistent with data obtained by *in situ* hybridization (Fig. 3). Mammalian GCAP1 is observed predominantly in cones and GCAP2 is found in both rods and cones (Cuenca *et al.*, 1998; Howes *et al.*, 1998; Kachi *et al.*, 1999). Our results strongly suggest that the distribution of these medaka GCAPs is quite different from those of mammalian GCAPs. Taking signal intensities and sample contents applied to a SDS-gel into account, we suggest that OlGCAP1 is present approximately 5 times as much as OlGCAP2 in ROS homogenates (Fig. 4a). This also agreed with data suggested by *in situ* hybridization. Based on these observation, we estimate that the molar ratios of OlGCAP1 and 2 to rhodopsin are about 1/200 and 1/1000, respectively, in medaka ROS. We also note that medaka rods have two kinds of GCs (OlGC-R1 and -R2) (Hisatomi *et al.*, 1999b); however, it is unclear now how OlGCAP1 and 2 regulate medaka GCs in rod photoreceptors. Recently, Mendez *et al.* (2001) evaluated the contribution of GCAPs to sensitivity regulation in rods by disrupting their expression in transgenic mice. It is concluded that Ca^{2+} feedback to GC via GCAPs strongly regulates the rod flash sensitivity under both dark- and light-adapted conditions, and that GCAP1 and GCAP2 may make distinct contributions to the regulation of GC in wild-type rods. Also, it is likely that OlGCAP1 and 2 may activate medaka GCs in different manners in rods.

Weak anti-O1GCAP1 reactivity was also found in the inner nuclear layer (INL) and ganglion cell layer (GCL) (Fig. 4b); however, anti-O1GCAP2 reactivity was absent in these layers (Fig. 4c). The immunopositive inner neurons are probably sub-populations of horizontal cells and ganglion cells (Fig. 4b, arrows). Because we could not identify *in situ* hybridization signals of OlGCAP1 in these layers, this reactivity may be due to cross-reaction of anti-OlGCAP1 antiserum to an OlGCAP1-like protein(s) expressed in these cells. Previous studies show that GCAP2, but not GCAP1, is distributed in horizontal cells, bipolar cells, amacrine cells and ganglion cells in mammalian retina (Cuenca *et al.*, 1998; Howes *et al.*, 1998). Thus, our observations strongly suggest that distribution of medaka GCAPs (or GCAP-like proteins) in other retinal cells is also different from that of mammalian GCAPs.

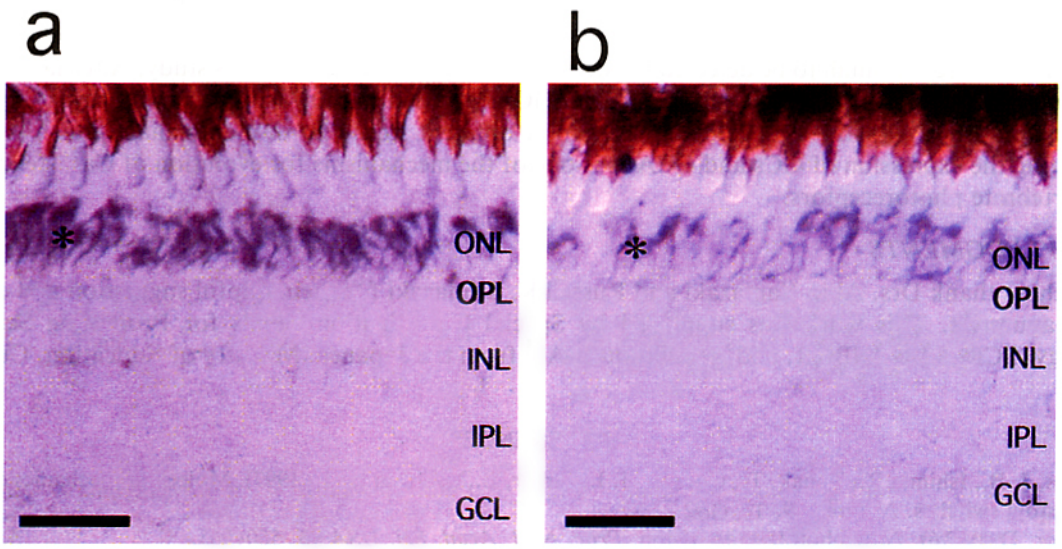

Fig. 3. Localization of OlGCAP1 (a) and OlGCAP2 (b) mRNAs in radial sections of medaka retina. Asterisks indicate the hybridization signals. Abbreviation: ONL, outer nuclear layer; OPL, ouetr plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar = 20 μm.

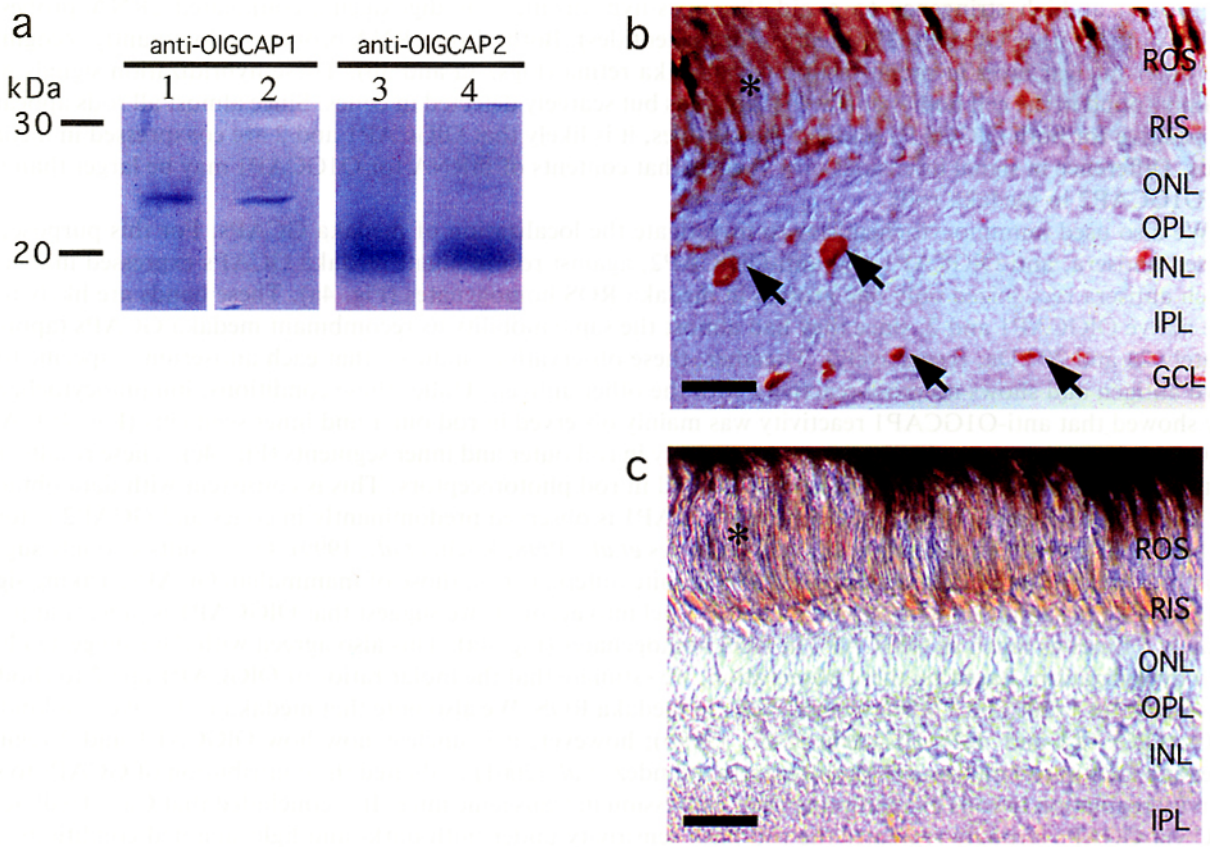

Fig. 4. Localization of GCAPs (or GCAP-like proteins) in medaka retina. (a) Western blot analysis of recombinant OlGCAP1 (2.5 ng) (lane 1) and medaka ROS homogenate (containing 1 μg rhodopsin) (lane 2) with anti-OlGCAP1 antiserum, and of recombinant OlGCAP2 (2 ng) (lane 3) and medaka ROS homogenate (containing 4 μg rhodopsin) (lane 4) with anti-OlGCAP2 antiserum. (b and c) Anti-OlGCAP1 (b) and anti-OlGCAP2 (c) immunoreactivities in the radial sections of medaka retina. Signals are found in the photoreceptor layer (asterisks), and in INL and GCL (arrows). Abbreviations: ROS, rod outer segment; RIS, rod inner segment; other abbreviations as in Fig. 3. Scale bar = 20 μm.

It has been suggested that medaka cone photoreceptors have at least one kind of GC (O1GC-C) (Seimiya *et al*, 1997; Hisatomi *et al.*, 1999b). However, we did not obtain positive signals of GCAPs in cones in the immunocytochemistry study. In the *in situ* hybridization study, as described above, we also did not obtain clear signals of medaka GCAPs in cones. Since under various conditions only two kinds of GCAPs were isolated from the medaka retinal cDNA library, we speculate that at least one of O1GCAP1 and 2 is present in cones but their contents in cones are too small to be detected by methods and antibodies used in this study. Alternatively, medaka cones may have a GCAP(s) totally different from GCAPs found in this study. Anyway, this study suggests that expression of GCAPs in teleost retina appears to be different from that of mammalian retina. We propose that changing the expression of GCs and GCAPs is one of the mechanisms for evolution of Ca^{2+}-feedback system in vertebrate photoreceptors.

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